Esp

Week 8

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

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1 PCR adding BamHI overhang to T7-EB

Responsible

Shuangjia Xue and Oskar Ohman

Protocols used

Q5 High-Fidelity Master Mix

Modifications and comments to protocols

Total reaction volume	12.5 μl
Primers used	VF-2F and EB-Rev

Experimental Data

Table 1: Set up of each reaction

Component	Volume [μl]	Final Concentration
Q5 High-Fidelity 2X Master Mix	6.25	1X
10μM forward primer	0.625	0.5μM
10μM reverse primer	0.625	0.5μM
Template DNA	0.25	20ng
Water	adjust to 12.5μl	-

Results and Conclusions

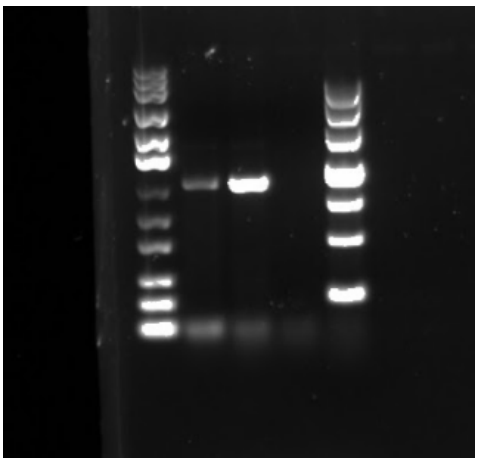

Figure 1: Result of the gel electrophoresis with 1 % agarose (1) DNA Ladder (2) EB1 (3) EB2 (4) Negative Control (5)DNA ladder.

The overhang PCR was successful as intense bands were observed in the gel. The concentrations of PCR product are 443.35 ng/μl and 464.8 ng/μl for EB1 and EB2. No contamination in negative control.

2 Digestion and ligation of T7-EB and linker-tag into pSB1C3 backbone. Transformation of ligation product

Responsible

Shuangjia Xue and Oskar Ohman

Protocols used

3A Assembly (Digestion and Ligation) Transformation

Modifications and comments to protocols

The pSB1C3 backbone and linker-tag are already digested. The T7-EB are digested with BamHI and EcoRI. No BSA is needed as the buffer provided is optimal for BamHI reaction. Transform the ligation into both TOP10 and BL21 cells.

Experimental Data

Table 2: Set up of each digestion reaction.

Samples	Volume [μl]
Purified EB overhang PCR product	4.4
Enzyme EcorI	1.8
Enzyme BamHI	1.8
Buffer B	0.8

Table 3: Set up of each ligation reaction.

Samples	Volume [μl]
Digested T7-EB	23.02
Digested linker-tag	1.11
Digested pSB1C3 backbone	4.35
T4 ligase	1
10x T4 DNA Ligase Buffer	3

Results and Conclusions

The ligation and digestion seems to be successful. However, no colony has developed after transformation.

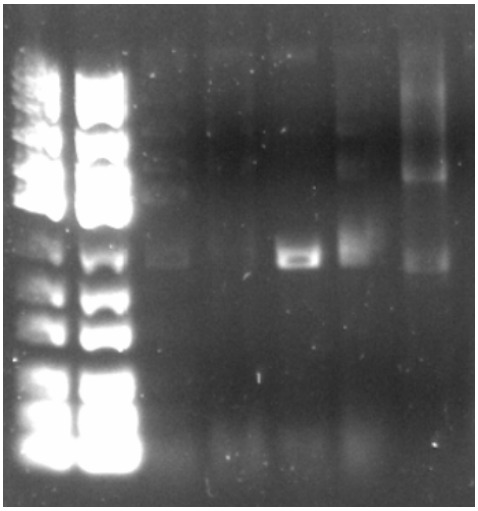

Figure 2: Result of the gel electrophoresis with 1 % agarose (1-2) DNA Ladder (3) irrelevant samples (4) EB1 digestion product (5) EB2 digestion product (6) EB1 ligation product (7)EB2 ligation product.

Discussion and Troubleshooting

We will try to re-ligate with another backbone sample and also try to re-transform the already existing ligaton product-sample.

3 Kirby-Bauer Test on EB

Responsible

Sigrun Stulz

Protocols used

None

Protocol description

Cellulose Nitrate disks were soaked for 10 minutes in either the samples listed above or cell lysis buffer or standard concentration of Kanamycin. On a plate of salt-free LB agar, 200ul of liquid E.coli Tob1 was streaked and subsequently the disks placed on the plate. The plate was incubated overnigh at 37C.

Results and Conclusions

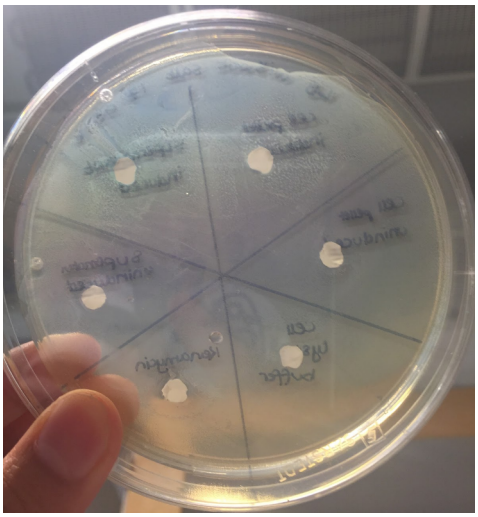

Figure 3: Kirby-Bauer test on EB

The growth of the bacteria was assessed. Only positive control (kanamycin) showed a halo.

Discussion and Troubleshooting

The positive control (kanamycin) showed a halo but it was difficult to distinguish because the agar was uncoloured. The other samples showed no inhibition which might be due to EB not efficiently inhibiting bacterial growth or also because the samples were frozen for a while in lysis buffer. Additionally, the filter paper that was used was nitrate cellulose paper which did not soak up the liquid very well. For a next test, coloured, selective agar (macconkey and Endo agar) will be used as well as either freshly produced protein and another kind of filter paper that will soak up the liquid more efficiently.

4 PCR adding BamHI overhang to truncated EB

Responsible

Sigrun Stulz

Protocols used

Q5 High-Fidelity Master Mix

Modifications and comments to protocols

Table 4: Primers

Sample	Forward primer	Reverse primer
T7-EB-pSB1K3	EB truncated forward	EB BamHI reverse
Positive control (T7-EB-pSB1K3)	VF2	VR2
Negative control (T7-Nuc-pSB1K3)	EB truncated forward	EB BamHI reverse

Experimental Data

Table 5: Set up of each reaction

Component	Volume [μl]
Water	18.5
10x PCR Buffer with MgCl	2.5
Nucleotides	1.5
Primers	1.25
polymerase	0.5

Results and Conclusions

The overhang PCR was successful as intense bands were observed in the right size of truncated EB and positive control. The concentrations of PCR purified product is 24.7 ng/μl and 464.8 ng/μl for truncated EB. There is also a band in negative control.

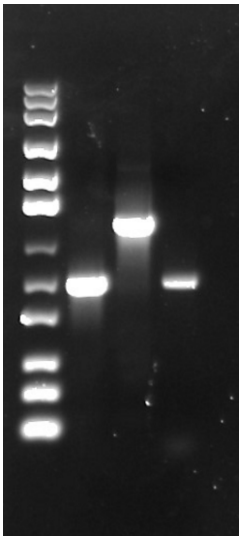

Figure 4: Result of the gel electrophoresis with 1 % agarose (1) DNA Ladder (2) Truncated EB (3) positive control (4) Negative Control.

Discussion and Troubleshooting

Theoretical size is 673 bp which seems to correspond to the length of the product visible on the gel =¿ primer seems to anneal strongly and specifically. In the Positive control - strong band of expected size, PCR seems to work nicely. In the negative control, there is still a band visible - either the primers anneal unspecifically even to Nuc and randolmy produce a fragment of the same size as truncated EB or Nuc was already at this point contaminated with EB.

5 Ligation of truncated EB into pSB1C3 backbone

Responsible

Sigrun Stulz

Protocols used

3A Assembly (Digestion and Ligation)

Experimental Data

Table 6: Set up of each reaction

Reagents	Volume [μl]
PCR purified product	6.07
Linker-tag	2.98
Backbone	8.33
T4 Ligase	1
T4 DNA ligase buffer	3
Water	8.61

Results and Conclusions

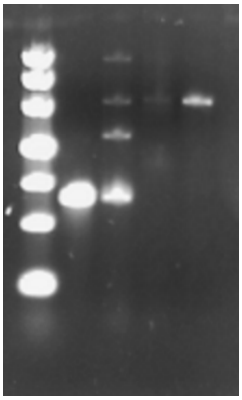

Figure 5: Result of the gel electrophoresis with 1 % agarose (1) DNA Ladder (2) Digested truncated EB (3) Ligated truncated EB (4) Irrelevant samples.

The ligation seems to have worked to a certain degree, as indicated by the two higher bands in the well with the ligation product. Transformation will be performed

6 Expression of pSB1K3-T7-EB

Responsible

Sigrun Stulz

Protocols used

Protein Expression Protocol

Summary

Four samples and two negative control were made: 1 mM IPTG at both 37C for 4h and 25C overnight, 0.5mM IPTG at 37C and 25h, neg. control without IPTG and neg. control as untransformed BL21 cells. Each sample was inoculated in 10ul LB for 4.5h at 37C to achieved OD600 = 0.6. Subsequently, 10μl or 5ul IPTG were added where appropriate.

7 SDS page for pSB1K3-T7-EB expression

Responsible

Shuangjia Xue and Oskar Ohman

Protocols used

SDS page Sonication Protocol

Modifications and comments to protocols

Sample preparation - Step 1:	Two batches of samples (28th Jul and 18th Aug)
Gel preparation - Step 2:	12% acrylamide of the separating gel
Electrophoresis - Step 3:	100 V for 90 min

Results and Conclusions

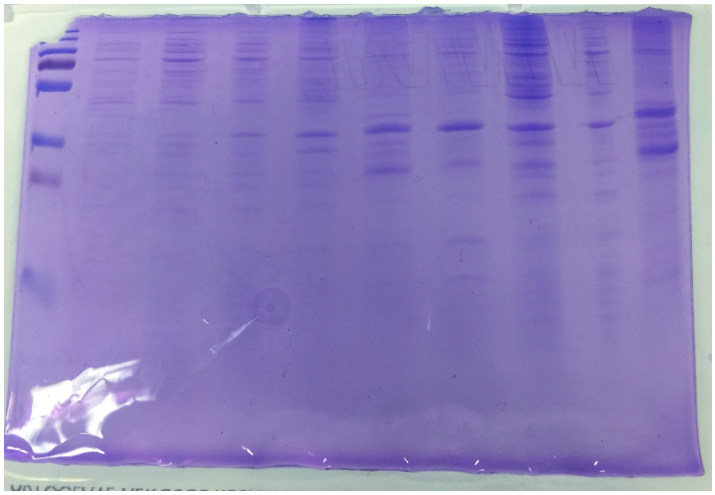

Table 7: Result of the 12% SDS page for protein prepared on 28th Jul.

Well	Sample	Result
1	Protein ladder	Well-separated ladder
2	EB uninduced soluble proteins	Too weak bands
3	EB 1μM IPTG soluble proteins	Too weak bands
4	EB 0.5μM IPTG soluble proteins	Too weak bands
5	EB uninduced insoluble proteins	Strong bands at around 35kDa
6	EB 1μM IPTG insoluble proteins	Strong bands at around 35kDa and 25 kDa
7	EB 0.5μM IPTG soluble proteins	Strong bands at around 35kDa
8	EB uninduced insoluble proteins (old samples)	Strong bands at around 35kDa and 25 kDa
9	EB induced soluble proteins	Too weak bands
10	EB induced insoluble proteins	Strong bands at around 35kDa and 25 kDa

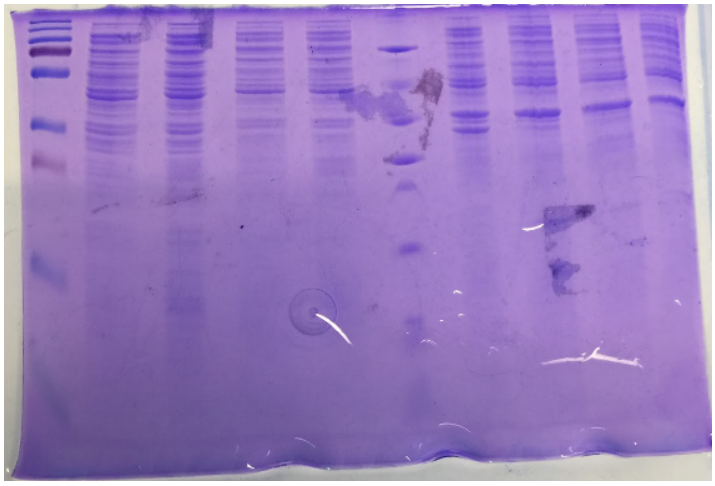

Table 8: Result of the 12% SDS page for protein prepared on 18th Aug.

Well	Sample	Result
1	Protein ladder	Well-separated ladder
2	BL21 untransformed soluable proteins	Strong bands at around 40kDa and 35kDa
3	EB uninduced soluble proteins	Similar as above
4	EB induced at 37 degree soluble proteins	Similar as above
5	EB induced at 25 degree overnight soluble proteins	Similar as above
6	Another ladder	Well-separated
7	BL21 untransformed insoluable proteins	Strong bands at around 40kDa, 35kDa and 33kDa
8	EB uninduced insoluble proteins	Strong bands at around 40kDa and 35kDa
9	EB induced at 37 degree insoluble proteins	Strong bands at around 40kDa and 35kDa
10	EB induced at 25 degree overnight insoluble proteins	Strong bands at around 40kDa and 35kDa

Discussion and Troubleshooting

The expression of pSB1K3-T7-EB seems to be successful for the first batch but not for the second one. EB is calculated to be 30 kDa and we observed an intense band around 30 kDa after IPTG induction. In the second SDS-page, we add the transformed BL21 cells to see whether there is a difference between the transformed and non-transformed cells. The result turns out the bands at 40kDa and 35kDa are universally expressed in all samples.

8 Transformation of Top10 and BL21 cells with pSB1A3-T7-EB-Linkertag. Liquid culture

Responsible

Sigrun Stulz

Protocols used

Transformation Colony picking

Summary

One single colony in TOP10 cells.

9 Ligate truncated EB and Linker-Tag into pSB1C3

Responsible

Sigrun Stulz

Protocols used

3A Assembly (Digestion and Ligation)

Experimental Data

Table 9: Set up of each reaction

Reagents	Volume [μl]
PCR purified product	6.07
Linker-tag	2.98
Backbone	8.33
T4 Ligase	1
T4 DNA ligase buffer	3
Water	8.61

Results and Conclusions

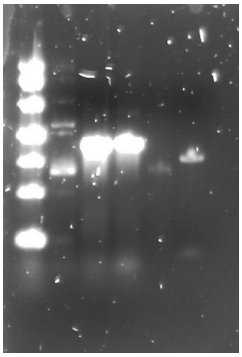

Figure 6: Result of the gel electrophoresis with 1 % agarose (1) DNA Ladder (2) Ligation product (3-4) Digested Truncated EB (5-6) Irrelevant smaples.

On lane two the Ligation product was loaded. Clearly, a band corresponding to digested PCR product can be identified as well as a longer band, likely corresponding to a ligation product. Therefore, transformation was attempted but on the next day yielded no colonies.